APPENDIX 1

ORAL CANCER DETECTION FROM AUTOFLUORESCENCE

A spin of result obtained during the course of our investigation on clinical trial of PDT was detection of oral carcinoma from the native fluorescence of tumour. Since there are various proliferative growths in the oral cavity and some of the growths mimic carcinoma, the detection of oral carcinoma is quite difficult. The conventional technique of biopsy is reliable but cumbersome. Often the patients develop phobia and become unco-operative for excision. This led many scientists to develop a non invasive technique to detect pathological changes, such as malignancy in tissues. In this context fluorescence emission spectroscopy has become an increasingly popular technique to probe the character of human tissue. For example, in PDT, when HPD is administered i.v., and the tumour was viewed under ultraviolet, the distinct reddish pink fluorescence of accumulated porphyrin could be seen. However, these extrinsic fluorescent markers are foreign agents and are known to interact with the normal native cellular environment [93]. Consequently, there is a need to develop new optical techniques to detect pathological changes in the malignant parts of tissues without affecting normal tissues. The use of such non invasive spectroscopic techniques are gaining important in recent time. Examples are: Alfano et al, [94] used autofluorescence to detect dental decay, which was later extended to detect cancer in rats and to the diagnosis of fibrous atherosclerosis [95,96]. Japanese scientists observed the yellow autofluorescence when gastric cancer was irradiated with Ar ion laser and suggested the
use for cancer diagnosis [97]. Using 365 nm line of the pulsed Xenon ion laser as the excitation source, Yang et al., observed the difference in the autofluorescence spectra between normal and cancerous lesion. The cancer tissues usually show characteristic peaks in the red region (630 nm and/or 690 nm) rather than yellow, which are absent in normal tissues [98]. We also tried to observe the autofluorescence from native carcinoma under the excitation of Nitrogen laser of wavelength 337.1 nm.

The patients for the present study were collected from Madras Dental college, Madras. These patients were classified into three groups as follows:

Group I  Patients with diagnosed and confirmed carcinoma (40 cases)
Group II Patients with suspected carcinoma (21 cases)
Group III Clinically diagnosed inflammatory growth(12 cases)

The lesion was examined clinically under the electrical lamp and the correct clinical diagnosis and provisional diagnosis was made. The patient was then allowed to sit on a chair comfortably. As the $N_2$ laser is a fixed one, the patients head was adjusted suitably, in such a way so that the oral mucosal lesion is focused to the beam of the $N_2$ laser. Care was taken to close the patients eyes with a sterile gauze bandage. The $N_2$ laser beam fall on the lesion and the surrounding structures of the oral cavity and careful observation was made to identify the fluorescence of normal tissues and the lesion. The observation was also recorded photographically. For that, a single lens reflex camera with large aperture lens fitted with Wratten 2B filter had been used to filter the UV light. A fast film of 1600 ASA was used, as the emission will be seen for a
fraction of seconds as they are excited by N₂ laser pulses. Figure A 1.1 shows the color of N₂ laser beam on a white paper.

In group I, among 40 patients reddish emission was observed for 37 cases only, whereas Histopathological reports showed that all 40 were having well differentiated carcinoma or undifferentiated carcinoma or anaplastic carcinoma. Thus the reliability is 95% with 3% false positive.

In Group II, among 21 cases seven showed red streak emission, indicating the malignancy and the remaining 14 cases showed only blue violet fluorescence indicating non-malignant, inflammatory growth (leukoplakia, chronic ulcers). The histopathological report indicated only 5 were found to have carcinoma and the remaining 16 were inflammatory growth. Thus, here again the reliability is about 90% with 10% positive result.

In Group III, the lesions of all the 12 patients showed blue violet fluorescence indicating non carcinomatic, inflammatory growth. This was fully confirmed with the histopathological report (100% reliability). Figure A 1.2 shows the appearance of leukoplakia and Figure A 1.3 shows the blue-violet emission under N₂ laser excitation indicating non carcinomatic condition.

A few important results based on our experience with these 73 cases are as follows:

i. Those lesion which exhibited distinct, fiery, charcoal red fluorescence later confirmed to be well differentiated carcinoma. Figure A 1.4 shows the
Figure A 1.1 Color of the nitrogen laser beam on the white paper
Figure A 1.2 Appearance of Leukoplakia

Figure A 1.3 Blue violet emission from lesion with nitrogen laser excitation
carcinoma at the lower lip

Figure A 1.4 Appearance of clinically dia
carcinoma at the lower lip

Figure A 1.5 Reddish emission from the lesion
the excitation of nitrogen laser
lesion before the illumination of Nitrogen laser and Figure A 1.5 shows the fiery charcoal red fluorescence from the lesion due to the excitation by the Nitrogen laser. This characteristic autofluorescence from the cancerous lesion may originate from the endogenous porphyrin which are accumulated at the lesion. This porphyrin are formed by the degradation of hemoglobin and they are localized and retained in the cancerous tissue [99].

ii. Those lesions which showed only mild streaks of red lines are confirmed later to be the early carcinoma.

iii. Those growth which showed pale blue green or bluish fluorescence are termed as non-carcinomatic. This includes leukoplakia, ulcers associated with the chronic irritation like fibrous epulis, pyogenic granuloma.

On the whole, considering the trial on 73 patients, we could say that oral carcinoma detection through autofluorescence under N_2 laser excitation, gives more than 90% reliability.

It is important at this stage to mention that in India, oral cancer has highest incidence mainly due to chewing tobacco, scented nuts and drugs like opium. The situation becomes aggravated since tobacco chewing is most common with lower middle class people, who have poor education, nutrition and unhygienic environment.

Since the Nitrogen laser can be home built and made portable, this autofluorescence technique is useful for mass screening of cancer in rural areas, in developing countries.